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Comparative biochemical analysis of haemolymph during metamorphosis of *Papilio Demoleus*

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ABSTRACT

The major constituents of plasma haemolymph like carbohydrates & lipids undergo quantitative changes during the development of an insect. In the present research the results of biochemical analysis of haemolymph reveals that various changes occur in the carbohydrate and lipid contents in the haemolymph of larva, pupa and adult of *Papilio demoleus*. Sugar level of 340 mg / 100 ml was found in the haemolymph of fifth instar larvae, and it was higher than sugar level in adults. While the lowest sugar level was observed in the eggs. Increased sugar level of each moult during larval developmental stages was correlated with the increased rate of food consumption & larval growth. Higher lipid level of 485.7 mg / 100 ml was found in the haemolymph of pupa, while it was lowest in the eggs. The female adult contained less lipid than the male adult. These changes in the composition of haemolymph indicate that various tissue specific changes occur during metamorphosis of *Papilio demoleus*.

Key words: Haemolymph, Metamorphosis, Carbohydrate, Lipid, Developmental stages.

1. INTRODUCTION

Insects and vertebrates share many common metabolic pathways. In many areas of research, insects are useful models that can facilitate our general understanding of biology. Investigations on insect haemolymph are of particular interest because they provide us with an adequate background to judge the synthetic activity associated with the different processes in developing organisms. Various changes occur in the biochemical composition of haemolymph during metamorphosis, particularly in holometabolous animals. Butterflies being holometabolous undergo profound biochemical changes in various tissues such as haemolymph and fat bodies, during different stages of development. The chemical composition of haemolymph is highly variable among the species and at different developmental stages of the same species.

Research with respect to quantitative & qualitative analysis of haemolymph, in various insects like *Drosophila*, *Periplanata*, *Bombyx mori*, *Calliphora* and other lepidopterans has been reported. Haemolymph of *Papilio demoleus* has not been studied so far, hence, in the present study, investigations have been carried out to evaluate the quantitative changes in lipids and carbohydrates of *Papilio demoleus* haemolymph.

In insects, as in other animals, sugars have a central place in metabolism. Carbohydrates are important biomolecules in insects, since they function as a major source of energy and for synthesis of fat and glycogen. They serve as a major fuel for growth and development of insects.

Kilby (1965) discussed carbohydrate metabolism in insect fat body. Chefurka (1965) recorded high sugar levels in insects as compared with those found in vertebrates. Usually in insects more than 0.5 percent sugar in blood was recorded however there were several records of more than 5 percent. In the aphid *Megouraviciae,* the larval haemolymph contained 13 - 20 mg / ml glucose that was greater than the adult (10 – 11 mg / ml) haemolymph. In solitary bees *C. japonica* still greater *i.e.* 30 - 48 mg / gm glucose was recorded (Kilby, 1965). The haemolymph of honeybee (*Apis mellifera*) contained 11 - 14 mg / ml of glucose (Gilmour, 1961), *Periplaneta americana* contained 7.2 mg / ml and in locust blood around 24 mg / 100 ml glucose was recorded (Chefurka, 1965).

The levels of reducing sugar in blood ranged in different species of insect between zero and 300 mg / 100ml, which, as Wyatt and Kalf (1967) remarked, appeared remarkably low for such a metabolically active animal.

A typical carbohydrate content in haemolymph appeared to reflect specialized diet. Hensen (1964) presented evidence that the blood of locusts fed on fructose rich food contains fructose but the blood of locusts fed on wheat does not contain fructose.

One of the prime impetus to study the lipid content in *P. demoleus* has been the finding that many insect growth hormones, pheromones and sex attractant are lipodal (Gilbert, 1967). Lipids are having vital importance in many insects as substrates for embryogenesis, metamorphosis and for flight. Several reviews on aspects of insect lipid chemistry and biochemistry have been appeared (Gilby, 1965) while other reviews considered the metabolism of lipid along with other biochemicals (Gilmour, 1961).

According to Gilbert (1967) there was wide variation in lipid content of insects belonging to different orders and even within a single family. Lipid content per individual larva was observed to increase with age and size. The newly hatched larva contained an appreciable amount of lipid, which increased as the larva grows. The prepupa loses water rapidly and lipid content increases, so that at pupation lipids constitute between 5 - 7 percent of the wet weight of the insect. At this time a sexual dimorphism in lipid content is evident, the male pupa contains 50% more lipid than the female (Niemierko, 1959).

In most insects the female contains more lipid than the male, as lipid is a most efficient substrate for egg development (Fast, 1964). However, the reverse may be true for many species as in the Lepidoptera. Gilbert and Schneiderman (1961) observed a sexual dimorphism in lipid content in the adult stage of *Hyalophora cecropia*. There was 50 % decrease in lipid content per individual between egg and first instar larva indicating lipid utilization during embryogenesis (Niemierko et al., 1956 and Rothstein, 1952).

2. METHODOLOGY

All laboratory experiments were carried out under standard condition of constant temperature of 24 ± 1°C & relative humidity of 75 ± 2 % & photo period of approximately 13 : 11 light : dark hours regime.

1) Estimation of sugar

Estimation of sugar (glucose) in the haemolymph of different developmental stages of *P. demoleus* was done by the Folin - Wu method (1920), with some modifications.

2) Estimation of lipid

Estimation of lipid in the haemolymph of different developmental stages of *P. demoleus* was done by the Frings and Dunn method (1970).

3. RESULTS AND DISCUSSION

The sugar and lipid content in the haemolymph of different developmental stages of *P. demoleus* were estimated.

1) Estimation of sugar

Sugar (glucose) was estimated in the haemolymph of different developmental stages of *P. demoleus*. Highest glucose level (340 mg / 100 ml) was found in the haemolymph of fifth instar larvae while the lowest sugar level was observed in the eggs.

Table 1

Level of Sugar in different developmental stages of *P. demoleus*

Sr. No.	Developmental Stage	Level of Sugar (mg/100ml)
1.	Egg	116.03
2.	First instar Larvae	243.18
3.	Second instar Larvae	236.85
4.	Third instar Larvae	293.64
5.	Fourth instar Larvae	312.16
6.	Fifth instar Larvae	340.52
7.	Pupa	218.47
8.	Female Adult	183.86
9.	Male Adult	197.07

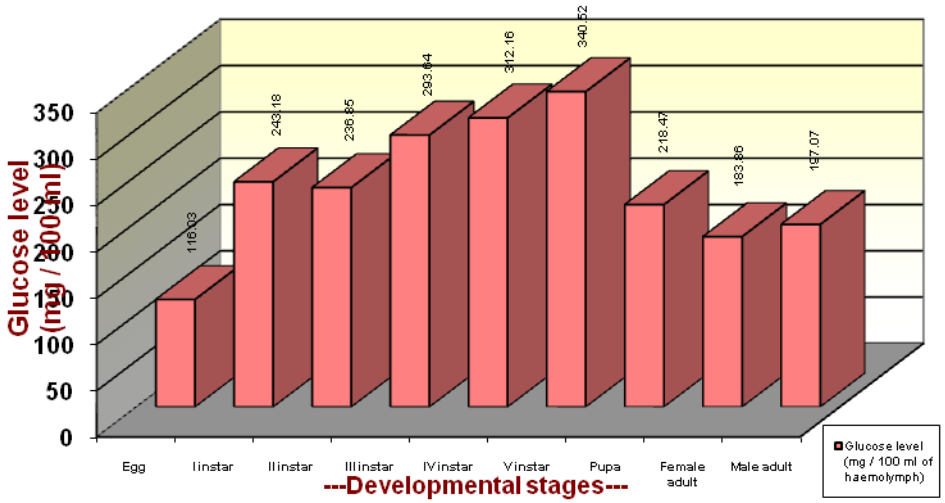

Figure 1

Level of Sugar in different developmental stages of *P. demoleus*

Kilby (1965) also reported that the larva of *Megouraviciae* contain higher levels (13 - 20 mg / ml) of glucose than the adult haemolymph (10 -11 mg / ml). Similar results were reported by Wyatt and Kalf (1967) that the glucose content decreases in the adult (24 mg / 100 ml) than in the larvae (200 mg / 100 ml) of *Schistocerca gregaria*. In contrast to our results, larva of *Phormiaregina* showed low glucose level (2 mg / ml) than the adult (6 mg / ml) (Chefurka, 1965).

2) Estimation of lipid

In present investigations lipid was estimated in the haemolymph of different developmental stages of *P. demoleus*. Higher lipid level (485.7 mg / 100 ml) was found in the haemolymph of pupa, while it was lowest in the eggs. The female adult contained less lipid than the male adult.

According to Gilbert (1967), lipid content per individual larva increases with age and size. The newly hatched larva contains an appreciable amount of lipid, which increases as the larva grows. The prepupa loses water rapidly and lipid content increases, so that at pupation lipids constitute between 5 - 7 percent of the wet weight of the insect.

During the pupal-adult transformation this dimorphism increased markedly until in the adult moth the tissues of males contain about 5 times as much lipid per gram fresh weight as the tissues of the female (Domroese and Gilbert, 1964).

Table 2

Level of Lipid in different developmental stages of *P. demoleus*

Sr. No.	Developmental Stage	Level of Sugar (mg/100ml)
1.	Egg	114.32
2.	First instar Larvae	132.46
3.	Second instar Larvae	153.48
4.	Third instar Larvae	242.03
5.	Fourth instar Larvae	372.26
6.	Fifth instar Larvae	456.25
7.	Pupa	485.70
8.	Female Adult	265.78
9.	Male Adult	317.85

The present findings are however very much similar with the observations of above mentioned workers.

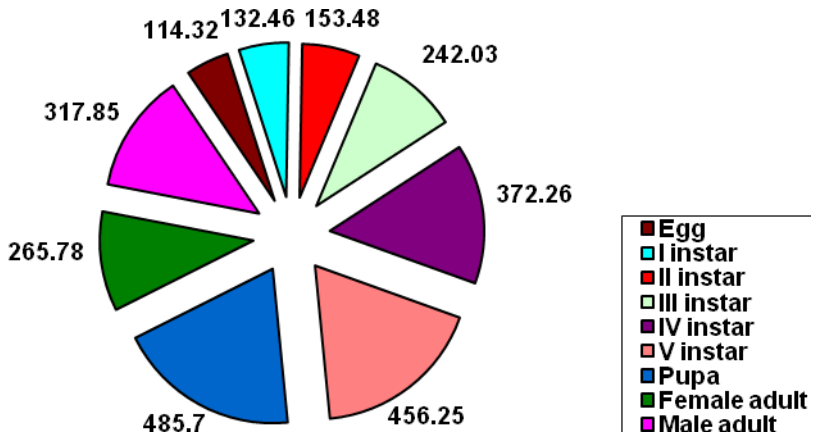

Figure 2

Level of lipid in different developmental stages of *P. demoleus*

4. CONCLUSION

Higher sugar level (340 mg / 100 ml) was found in the haemolymph of fifth instar larvae than adults of *Papilio demoleus*, while it was lowest in the eggs of *Papilio demoleus*. Increased sugar level of each moult during larval developmental stages can be correlated with the increased rate of food consumption & larval growth. It may also be stated that the insect conserve sufficient amount of energy reserves during larval stage to meet energy requirements at pupal and adult stages. During pupa formation, water is lost rapidly hence highest lipid level (485.7 mg / 100ml) was found in the haemolymph of pupa, while it was lowest in the eggs. The female adult contained less lipid than the male adult as lipids are used as substrate during flight and also for the formation of eggs.

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